

CONCISE COMMUNICATIONS

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Investigation of linkage on chromosome 2q and hand and knee osteoarthritis

Osteoarthritis (OA) is a heterogeneous disorder in terms of etiology and outcome and is the most common condition to affect synovial joints in humans. Although OA may result from direct injury or be associated with rare inherited skeletal dysplasias, it is in most instances a “primary,” late-onset multifactorial condition. There is increasing evidence that genetic factors contribute to OA susceptibility and that genetic influences may vary according to the site and pattern of joint involvement and sex. In particular, twin studies of radiologic OA in women have shown heritabilities for hand and knee OA ranging from 39% to 65% (for review, see ref. 1). Such findings have prompted more studies to help identify the underlying OA susceptibility genes.

Of the studies conducted thus far, 4 independent groups have reported linkage and/or association with OA on chromosome 2q. The most comprehensive study involved the finer mapping of a region of 2q (previously identified from a genome scan [2]) by typing 378 families from the Oxford region of the UK that each contained at least 1 affected sibling pair (ASP) concordant for primary hip OA (3). This study mapped the hip OA locus to an 8.6-cM region of 2q24.3–q31.1 with a maximum multipoint logarithm of odds (LOD) score (MLS) of 1.6 between markers D2S2330 and D2S326. We previously reported evidence of linkage to 2 markers (including D2S326) within the above locus in a study of 66 nodal OA ASPs from the Nottingham area of the UK (4) and also in a genome scan including 29 of these families plus 61 other families from the same geographic region (5). A genome scan involving 27 Finnish families with severe radiographic distal interphalangeal (DIP) joint OA identified 4 genomic regions of linkage, 1 of which was to 2q12–q13, with an MLS of 2.34 (6). A study of 4 families from the Netherlands with autosomal-dominant forms of early-onset generalized nodal OA identified 2 regions of linkage on chromosome 2q, 1 of which overlaps with that of the Oxford hip OA locus and 1 with the Finnish DIP OA locus (7). Also, association between the interleukin-1 gene cluster and knee OA has been reported (8).

In light of these findings, we conducted a detailed linkage study across the 2q11.2–q36.3 region by typing members of a total of 410 white families, each containing at least 1 ASP, all from the Nottingham area. These included members of the 90 nodal OA families discussed above plus another 110 nodal OA families, comprising a total of 350 ASPs. In addition, 210 knee OA families, comprising 300 ASPs, were typed. Using previously published formulas (9), we estimated that both the nodal OA and knee OA cohorts have 80% power to detect a locus with λ_s of 1.2 at a 5% significance level. Ethical approval for the study was obtained from the Research Ethics Committee of Nottingham City Hospital. All subjects were 40 years of age or older.

Nodal OA was defined clinically by the presence of multiple, bilateral Heberden's (and/or Bouchard's) nodes affecting at least 2 digits of each hand in the absence of overt trauma, with radiographic confirmation of interphalangeal OA (osteophyte plus narrowing) and the absence of nonproliferative or proliferative marginal erosion. Knee OA was defined radiographically by the presence of definite osteophytes plus definite joint space narrowing in at least 1 compartment

(medial tibiofemoral and/or patellofemoral) of at least 1 knee, seen on standardized standing anteroposterior (fully extended) and skyline (30° flexion) views.

In addition to defining OA at the primary site (hand or knee), clinical and radiographic evidence for OA at other sites was assessed wherever possible. This allowed the stratification of the nodal OA families into 2 subsets: 150 families comprising 260 ASPs concordant for generalized nodal OA (GNOA; defined as nodal OA plus evidence of OA at another site) and 67 families comprising 100 ASPs concordant for nodal and knee OA. Only a small proportion of the nodal OA ASPs were also concordant for hip OA (26 families comprising 30 ASPs) or also concordant for hip and knee OA (8 families comprising 10 ASPs), preventing stratification of the nodal OA data according to these criteria. The nodal ASPs comprised 238 female-concordant pairs and 12 male-concordant pairs, and the knee ASPs comprised 116 female-concordant pairs and 67 male-concordant pairs.

To cover the 2q11.2–q36.3 region, family members were typed for 24 microsatellite markers with an average spacing of 5 cM. The nodal OA samples were typed using an ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA) and the associated Genescan 3.1 and Genotyper 2.1 software (Applied Biosystems). The knee OA families were typed using a MegaBACE 500 DNA sequencer (Amersham, Little Chalfont, UK) and the associated Genetic Profiler 1.1 software (Amersham). Hence, the nodal and knee OA data were analyzed separately. Genotyping errors and misinheritances were detected using GAS 2.0 (<http://users.ox.ac.uk/~ayoung/gas.html>). Allele frequency calculations and single-point linkage analysis were carried out using SPLINK 1.09 (<http://www.mrc-bsu.cam.ac.uk/pub/methodology/genetics>). Multipoint nonparametric linkage analysis was performed using Genehunter Plus 1.2 (<ftp://galton.uchicago.edu/pub/kong>).

The results of the multipoint linkage analysis are summarized in Figure 1. Analysis of the genotype data for the 200 nodal OA families provided a maximum nonparametric linkage (NPL) score of 0.34 (LOD 0.04, $P = 0.37$) in the region of marker D2S142 and -0.24 in the region of D2S326. Stratification of these data for GNOA generated a maximum NPL score of 0.14 (LOD 0.01, $P = 0.44$), again in the region of D2S142, and stratification for nodal plus knee OA generated a maximum NPL score of 1.02 (LOD 0.47, $P = 0.13$) in the region of D2S364. Analysis of the genotype data from the 200 knee OA families produced a maximum multipoint NPL score of -0.55 (LOD 0.00, $P = 0.71$) in the region of D2S2299. Previous linkage analyses have shown possible sex-specific effects, with a greater effect in women. When we stratified the families in our study into female-concordant ASPs, a similar lack of significant linkage was found for both the nodal and knee OA cohorts (data not shown). The data were not stratified into male-concordant ASPs because of the small sample sizes.

The absence of linkage to 2q in the 200 nodal OA families (including the subsets of GNOA and nodal plus knee OA) and the 210 knee OA families provides evidence against the presence of a major susceptibility locus for these forms of OA in this region. The lack of linkage to nodal OA is supported by a recent genome scan for hand OA (10) that also showed no evidence of linkage to chromosome 2q, although a multipoint LOD score of 2.23 was found at 2p23.2. It is likely that the positive LOD scores obtained in our original studies represented false-positive results due to the small sample size and the resulting bias from a few outlying families. Also,

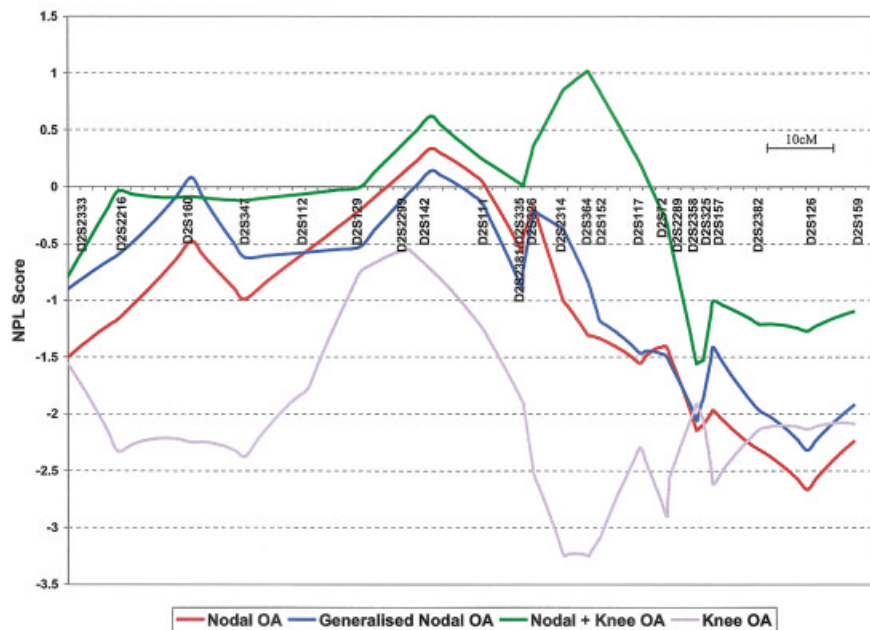


Figure 1. Multipoint linkage analysis of 24 microsatellite markers from chromosome 2q11.2–q36.3 and osteoarthritis (OA).

differences between the results of our study and those reported from other groups may reflect similar limitations in study design, differences in patient ascertainment and categorization (for example, the knee OA in our study was diagnosed radiographically, whereas the patients with knee OA in ref. 8 had all undergone knee replacement surgery), and the underlying heterogeneity of OA.

The results of the current study have underlined the fact that there are as yet no confirmed loci for idiopathic forms of OA. Moreover, it is becoming increasingly clear from existing genetic and epidemiologic evidence (11) that the genetic risk factors for OA at different joint sites may well be distinct, making the unmasking of susceptibility genes even more problematic.

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Humanized monoclonal anti-interleukin-6 receptor antibody for treatment of intractable adult-onset Still's disease

Adult-onset Still's disease (AOSD) is a clinical syndrome of unknown etiology, characterized by spiking fever, salmon-pink rash, and articular symptoms (1). It has been reported that serum levels of interleukin 6 (IL-6) are markedly elevated in patients with AOSD only during an active phase (2,3). Overproduction of IL-6 could account for major symptoms in AOSD, because it induces fever, leukocytosis, thrombocytosis, acute-phase protein production, and bone resorption (4). We administered a humanized anti-human IL-6 receptor (IL-6R) monoclonal antibody of the IgG1 subclass to a patient with intractable AOSD and severe osteoporosis, based on the assumption that overproduction of IL-6 was the major cause of AOSD.

The patient, a 23-year-old Japanese woman, was diagnosed with AOSD in July 1998 based on the presence of fever, evanescent rash, and arthralgia, according to the criteria described by Yamaguchi et al (5). Treatment was started with prednisolone (50 mg/day), indomethacin (75 mg/day), and intramuscular sodium aurothiomalate (10 mg every 2 weeks), with favorable effects on both systemic and articular symptoms. The gold injections were successfully discontinued when the total amount administered reached 40 mg.

Over the next 18 months, the patient's disease exacerbated and remitted repeatedly, and flares recurred when the dosage of prednisolone was reduced to 9–20 mg/day. In January 2000, she experienced high fever and arthralgia. Methotrexate therapy was initiated, and the dosage was increased to 15 mg per week, with little effect. The addition of cyclosporin A (4 mg/kg body weight) to methotrexate, along with an increased dosage of prednisolone (to 50 mg/day) did not induce any appreciable effects. Double-filtration plasmapheresis, which was performed only once, was not effective. In May 2000, severe osteoporosis developed in the patient's lumbar vertebrae, attributable to prolonged use of high-dose prednisolone. In June 2000, multiple bone fractures of thoracic vertebrae and ribs, causing intractable pain, made it impossible for her to breathe by herself, whereupon she was placed on a respirator in the intensive care unit. At that time, her serum level of C-reactive protein (CRP) increased to 438 $\mu\text{g/ml}$. Infections having been ruled out, she received an infusion of betamethasone 100 mg/day for 3 days, followed by betamethasone 4 mg/day or dexamethasone 4 mg/day. This treatment alleviated the activity of AOSD, and her serum CRP level decreased to 32 $\mu\text{g/ml}$.

Because high-dose glucocorticoids had to be avoided to prevent further aggravation of her osteoporosis, a humanized anti-human IL-6R monoclonal antibody (MRA; Chugai Pharmaceutical, Tokyo, Japan) was considered as a therapeutic option. MRA suppresses the biologic activity of IL-6 and is now being used in clinical trials for rheumatoid arthritis (RA) in Japan and Europe. The ethics committee of our hospital approved the use of MRA, and informed consent was obtained from the patient and her family members.

Before the administration of MRA, her serum IL-6 level was 403 pg/ml (normal <4). After she had received a

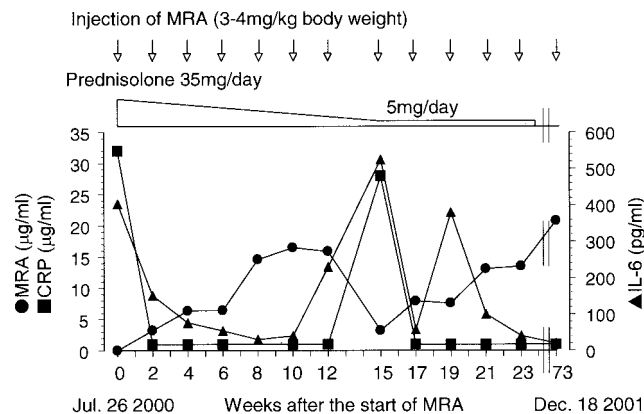


Figure 1. Serum levels of C-reactive protein (CRP), interleukin-6 (IL-6), and MRA. MRA very rapidly suppressed CRP and IL-6 levels. When the infusion cycle was extended from once every 2 weeks to once every 3 weeks at week 15, levels of both CRP and IL-6 increased.

single dose of MRA (4 mg/kg body weight), her serum CRP level decreased rapidly, from 32 $\mu\text{g/ml}$ to 1 $\mu\text{g/ml}$ in a week, along with defervescence and improvement of arthralgia (Figure 1). When the interval between infusions was extended from once every 2 weeks to once every 3 weeks, her CRP level increased to 28 $\mu\text{g/ml}$, and her left shoulder became swollen. Therefore, the infusion schedule was returned to once every 2 weeks. The dose of MRA was reduced, from 4 mg/kg to 3 mg/kg, beginning with the seventh infusion and thereafter. By December 2001, a total of 37 infusions of MRA had been administered.

The dramatic improvement in both systemic and articular symptoms achieved with MRA enabled withdrawal of prednisolone, without flares. MRA did not induce any serious side effects, such as severe infection, or any local reactions during 17 months of the treatment. Antibodies against MRA did not develop. Hypocomplementemia, which was probably due to the consumption by immune complexes, was detected in the absence of any lupus-like manifestations; an increased level of soluble IL-6R complexed with MRA was observed in a pilot study on patients with RA (Nishimoto N: unpublished observations). At present, rheumatoid factor and anti-DNA antibodies are not detectable in the patient, although antinuclear antibodies became weakly positive (1:20 serum dilution). Serum IL-6 levels stayed slightly elevated, at ~20–30 pg/ml, during MRA treatment, possibly because of a compensatory increase in IL-6 production.

In theory, there are 2 means of blocking IL-6 signal: antibodies against IL-6R, and the soluble form of IL-6R. Unlike soluble tumor necrosis factor (TNF) receptor, which blocks TNF signal, soluble IL-6R can even augment IL-6 signal by binding to the signal-transducing gp130.

Inasmuch as IL-6 is proposed as one of the major pathogenic factors in RA and AOSD (2–4), IL-6 antagonists such as MRA would be a powerful therapeutic modality by suppressing the activities of IL-6. AOSD is a rare but potentially devastating disease and sometimes demands use of toxic, second-line agents, as well as high-dose and/or long-term

systemic glucocorticoids. Therefore, MRA deserves further evaluation in extended clinical trials.

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